The Phytanic Acid Content of the Lipids of Bovine Tissues and Milk

A.K. LOUGH, Rowett Research Institute, Bucksburn, Aberdeen, Scotland, AB2 9SB

ABSTRACT

In three steers which were given grass silage for six months, the content of phytanic acid (i.e. 3,7,11,15-tetramethylhexadecanoic acid) in plasma lipid increased to about 8% of the total fatty acids, whereas after this time the proportion in the total fatty acids of liver and heart lipids was about 1%, and only 0.1% in those of kidney lipids; the acid was present in trace amounts in adipose-tissue triglycerides and was apparently absent from brain lipids. In eight lactating cows which were given grass silage for about 3 months, the content of phytanic acid in the total long chain fatty acids of milk and of plasma was 0.7% and 13%, respectively. In the plasma lipids of both steers and lactating cows, phytanic acid constituted a substantial proportion of the total fatty acids of the triglycerides and phospholipids; the acid was present in lowest proportion in the cholesteryl esters.

INTRODUCTION

The multibranched fatty acid, phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a normal constitutent of the lipids of the plasma, tissues, and milk of ruminant animals (1). The carbon skeleton of the acid is of exogenous origin and in herbivores is apparently largely derived from phytol, the alcohol moiety of chlorophyll (1,2). In cattle, the proportion of phytanic acid in the total fatty acids of plasma lipids was found to vary widely depending on the composition of the feed ingested (1). Phytanate was present in abundance in the plasma lipids of cows given grass silage (1) and comprised up to 40% of the fatty acids of plasma phospholipids isolated from a cow which had been given ensiled grass (3).

In rats, mice, rabbits, and chinchillas given experimental diets that were rich in phytol or phytanic acid, the multibranched acid was found to accumulate, not only in the plasma lipids, but also in tissue lipids, notably those of kidney, liver, and heart (4-6). It was therefore decided to investigate the lipids of the plasma, tissues, and milk of cattle in relation to the accumulation of phytanate which can be induced when they are fed on grass silage; some observations were also made on phytanic acid in the lipids of maternal and foetal plasma.

METHODS AND EXPERIMENTAL PROCEDURES

Lipid Extraction

Plasma samples were freeze-dried, and water amounting to one-fifth of the original volume of plasma was added to the residue. The partially reconstituted plasma was extracted with chloroform-methanol (2:1 v/v) as described by Folch et al. (7). Tissue samples which had been freshly excised or which had been stored at -20 C were also extracted by the method of Folch et al. (7) as were the samples of milk lipid that were obtained by centrifugation of freshly-collected milk.

Lipid Fractionation

Lipids were separated by silicic acid chromatography into fractions comprising cholesteryl esters, triglycerides, and phospholipids; unesterified fatty acids were isolated by treatment of the neutral lipids with aqueous KOH according to Garton et al. (8). Methyl esters from each lipid fraction were prepared as previously described (9).

Gas Chromatography

The analysis of methyl esters of long chain fatty acids was made using Apiezon L and ethyleneglycol-adipate polyester (EGA) as stationary phases (9). Since methyl phytanate is superimposed upon heptadecanoate when eluted from EGA and by octadecadienoate and octadecatrienoate on the nonpolar phase, determination of the multibranched acid was made on Apiezon L after removal of the unsaturated fatty acid esters using mercuric acetate or by their prior conversion to saturated esters by catalytic hydrogenation (1).

Experiment A

Three Friesian steers which from weaning had been reared on barley supplemented with hay and dried grass, were given grass silage only for a period of about 6 months, after which they were killed. Blood samples were taken at the start of silage-feeding and on three subsequent occasions; the final sample was taken 3

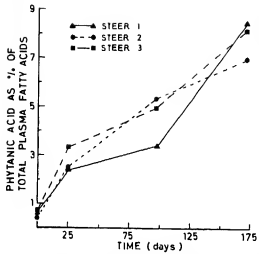

FIG. 1. Changes in the proportion of phytanic acid in the total fatty acids of the plasma lipids of three steers given grass silage to appetite.

days before slaughter. Tissue samples were taken immediately after slaughter and were stored at -20 C for not longer than 4 wk before they were analyzed.

Experiment B

Samples of milk and of plasma were taken from eight Friesian cattle which were in their first lactation and which had been given grass silage only for about three months. Three of the eight samples of plasma lipids were fractionated; five samples were examined without prior fractionation.

Experiment C

Two pregnant Friesian heifers were given grass silage supplemented with concentrates comprised of barley, oats, and linseed meal. After parturition, blood samples were taken from the calves before they were suckled. Blood samples from the heifers were taken at calving.

The animals used in Experiments A and C were from the Duthie Experimental Farm, Rowett Research Institute, Aberdeen, while those used in Experiment B were from Grange Farm, County Meath, The Agricultural Institute, Ireland.

RESULTS

The changes in the content of phytanate in the plasma lipids of the three animals in Experiment A are shown in Figure 1 which indicates that, within 4 wk of the commencement of silage feeding, the proportion of the multibranched acid had increased markedly. The phytanate content of plasma lipids continued to increase throughout the experiment and was apparently still increasing at the time the final blood sample was taken. At slaughter the values for the proportion of phytanic acid in the total fatty acids of plasma were 6.9, 8.2, and 8.4%.

The composition of the fatty acids of the main lipid classes and the contribution which the fatty acids from each class made to the plasma total fatty acids of the plasma samples obtained 97 and 171 days after the commencement of silage feeding are shown in Table I.

In agreement with previous findings on bovine plasma (3), phytanic acid was distributed throughout the main lipid classes. In the present investigation, the proportions of phytanate which contributed to the total fatty acids of the triglycerides and of the phospholipids, respectively, were similar, though in two of the blood samples which were taken at 97 days, the proportion of phytanate in glyceride fatty acids exceeded that in the phospholipid fatty acids. The proportion of phytanic acid in the unesterified fatty acids was considerably lower than in the glyceridic lipids, while only traces were found in the cholesteryl ester fraction. It should be noted that, with time, the proportion of phytanic acid present in any given lipid class may change depending on the turnover time of the phytanyl residues in relation to their site of esterification within glycerolipid molecules. Given a fairly constant intake of phytol, it is possible that an "equilibrium state," with respect to lipid classes in a particular organ, might be reached, but this was probably not the case in the present experiment in which continuously increasing levels of phytanate were observed in plasma total lipids.

The proportions of phytanate in the component fatty acids of tissue lipids are given in Table II, which shows that phytanate was present as a minor constituent in the lipids of liver, kidney, and heart. Table II also shows that phytanate was only a trace component of the fatty acids of omental depot lipids, and was apparently absent from the lipids of brain. For the most part, the phytanate content of the fatty acids of the main lipid classes of the tissues was less than 1%; only the triglycerides of liver contained appreciable amounts (up to 8%).

The mean values for the proportion of phytanate in the total fatty acids of the lipids of liver and heart were 1.2% and 1.1% respectively, so that about a sevenfold difference existed between these values and the corresponding proportion (7.9%) of phytanate in the plasma total fatty acids.

TABLE I

Content of Phytanic Acid in the Main Lipid Classes of Plasma from Three Steers Given Silage[a]

	Steer 1		Steer 2		Steer 3	
Days in silage	97	171	97	171	97	171
Cholesteryl esters	tr[b] (42.9)	tr (41.6)	tr (37.9)	tr (39.3)	tr (43.4)	tr (38.4)
Triglyceride	11.5 (3.3)	14.4 (7.0)	9.1 (6.7)	8.4 (6.7)	13.7 (4.9)	8.9 (8.1)
Unesterified fatty acid	2.1 (5.2)	2.9 (1.7)	1.8 (4.9)	0.7 (3.4)	0.9 (13.5)	2.5 (2.5)
Phospholipid	6.0 (48.6)	14.8 (49.7)	9.1 (50.5)	12.5 (50.6)	10.7 (38.2)	14.5 (51.0)

[a]Values expressed as percengage of total fatty acids; the percentage contribution of the fatty acids of each lipid class to the total fatty acids, is given in parentheses.

[b]tr = trace amount (i.e. <0.2%)

TABLE II

Content of Phytanic Acid in the Main Lipid Classes of Liver, Kidney and Heart, and in the Total Lipids of Brain and of Omental Depot Tissues, of Three Steers Given Grass Silage for 174 days[a]

Tissue	Lipid	Steer 1	Steer 2	Steer 3
Liver	Cholesteryl ester	ND[b] (2.8)	1.7 (2.1)	ND (4.8)
	Triglyceride	7.9 (2.6)	3.7 (9.5)	2.2 (14.3)
	Unesterified fatty acid	0.4 (11.4)	0.7 (4.3)	0.5 (1.8)
	Phospholipid	0.9 (83.2)	1.8 (84.1)	0.4 (79.1)
Kidney	Cholesteryl ester	tr[c] (4.2)	tr (3.4)	ND (4.6)
	Triglyceride	0.4 (6.7)	0.4 (1.9)	0.3 (1.7)
	Unesterified fatty acid	ND (10.8)	ND (24.9)	tr (17.4)
	Phospholipid	0.3 (78.3)	ND (69.2)	ND (76.3)
Heart	Cholesteryl ester	tr (6.1)	1.0 (4.7)	1.9 (5.8)
	Triglyceride	2.1 (16.4)	0.7 (26.3)	2.7 (33.4)
	Unesterified fatty acid	tr (8.6)	tr (5.5)	0.4 (5.8)
	Phospholipid	0.4 (68.9)	0.5 (63.5)	2.3 (55.0)
Brain	Total	ND	ND	ND
Omental depot	Total	ND	tr	tr

[a]Values expressed as percentage of phytanate in total fatty acids in each lipid class, or in total lipid, the percentage contribution of the fatty acids of each lipid class to the total fatty acids, is given in parentheses.

[b]ND = not detected

[c]tr = <0.2%

TABLE III

Content of Phytanic Acid in the Total Long Chain Fatty Acids of Plasma and of Milk of Eight Lactating Cows Given Grass Silage; the Proportion of Phytanate Contributing to the Total Fatty Acids of the Main Lipid Classes of the Plasma Lipids of Three of the Eight Animals[a]

	Plasma lipids					Milk lipids
	Cholesteryl ester	Triglyceride	Unesterified fatty acid	Phospholipid	Total	Total
Cow	1.1 (35.2)	15.2 (8.4)	7.6 (1.0)	23.9 (55.4)	15.0	1.0
Cow	0.9 (39.3)	18.0 (4.5)	8.3 (1.3)	20.9 (54.9)	12.7	0.5
Cow	0.6 (40.8)	17.7 (5.3)	8.1 (1.3)	22.1 (52.6)	12.9	0.8
Mean of 8 cows					13.2 ± 1.4	0.69 ± 0.19

[a]Values expressed as percentage of total fatty acids; the percentage contribution of the fatty acids of each lipid class to the total fatty acids is given in parentheses.

The results of the analysis of blood-plasma lipids and of milk lipids obtained in Experiment B are shown in Table III; the values respresenting the proportions of phytanate in milk lipids are expressed in terms of total long chain fatty acids (i.e. acids of chain-length $>C_{12}$) and are

therefore about one-third greater than might be expected from calculations based on the entire complement of fatty acids of these lipids (10).

From Table III, it can be seen that, whereas phytanic acid accounted for up to 15% of the total fatty acids of plasma, it was present only as a minor constituent of the fatty acids of milk. Seven of the eight samples of milk had a phytanate content that was less than 1% of the total long chain fatty acids. Comparing the mean values shown in Table III, the extent of the disparity in the proportions of phytanate in the fatty acids of plasma and of milk is about twentyfold. A similar order of difference also exists between the proportion of phytanate in the fatty acids of the plasma triglycerides and that in the long chain fatty acids of milk fat.

With regard to the content of phytanate in the main lipid fractions of plasma (Table III), it can be seen that the pattern of distribution of the multibranched acid is closely similar to that observed in the experiment in which steers were used (Table II). Thus, in the sample of plasma taken after 171 days on experiment, the phospholipid fraction contained the major proportion of the total plasma phytanate. Furthermore, the contribution of phytanate to the total fatty acids of each lipid fraction was greatest in the phospholipid class.

Results obtained in Experiment C showed that, whereas the proportion of phytanic acid in the total plasma lipids of maternal plasma were 5.6% and 7.4% phytanic acid was not detected in the total fatty acids of calf plasma.

DISCUSSION

The finding of phytanate in high proportions in the plasma lipids of cows given grass silage confirms a previous observation (3). In addition, it was found that the multibranched acid was present in greatest proportion in the fatty acids of phospholipids and occurred in smallest proportion in the fatty acids of the cholesteryl esters, thus exhibiting a pattern of distribution between the lipid classes which was similar to that reported in earlier work (3).

In the experiment using steers, the presence over some months of phytanate in substantial proportion in the triglycerides and phospholipids of plasma was not associated with its deposition in quantity in any of the tissues studied. The virtual absence of phytanic acid from brain lipids is especially noteworthy because these lipids have been the subject of considerable interest in connection with the possible relationship between the occurrence of the acid in nervous tissue lipids and the neuropathology of Refsum's Syndrome in man (1).

The lack of phytanate in the adipose tissue lipids of the steers is perhaps not surprising since experiments which involved the feeding of phytol, dihydrophytol, or phytanic acid to rats resulted in the assimilation of only very small amounts of phytanate into depot lipids, while marked accumulation of the acid in the lipids of kidney, liver, and heart occurred (4,5). Because there is considerable evidence to suggest that triglycerides are hydrolyzed to unesterified fatty acids during their uptake from plasma into adipose tissue (11,12), it is probably of relevance that the activity of lipoprotein lipase towards glycerides containing phytanyl groups is considerably lower than that towards glycerides composed of normal acids. Thus, Laurell (13) observed that lipoprotein lipase was inactive towards glyceryl triphytanate, whereas the enzyme readily hydrolyzed glyceryl tripalmitate. Later, Ellingboe and Steinberg (14) confirmed Laurell's findings and further showed that there was virtually no hydrolysis of glyceryl 1,3-diphytanyl-2-palmitate, but that there was significant hydrolysis of the phytanyl ester bond of triglycerides containing one phytanyl group in either the 1- or 2-position. While it would seem unlikely that triglycerides containing two or three phytanyl groups were present to any extent in the plasma lipids of the cows and steers used in the present experiments, there would appear to be little doubt that, as previously suggested (13,14), the low proportions of phytanic acid in the depot lipids are to some degree the result of the inhibitory action of the branched chain structure of the acid towards lipoprotein lipase.

The low activity of lipoprotein lipase towards phytanyl ester bonds of glycerides may also be of significance in relation to the observation that the phytanate content of milk lipid is low compared with that of plasma triglycerides. It is known that triglycerides are taken up from plasma by the cow mammary gland and that fatty acids which are released from ester combination enter a metabolic pool before they are incorporated into milk lipids (15). Since almost the entire complement of C_{18} fatty acids in cow milk fat is derived from plasma glycerides (15), the marked difference in the proportion of phytanic acid between plasma glycerides and milk lipids would suggest that there exists a high degree of selectivity against the incorporation of phytanic acid into milk lipids. While it is likely that this selectivity is due to the relative inactivity of hydrolytic enzymes towards phytanyl ester groups, other

conditions may also be contributory. Thus, in their consideration of factors influencing the deposition of phytanate in adipose tissue, Ellingboe and Steinberg (14) suggested that the kinetics of enzymes catalyzing the esterification and transesterification of phytanic acid might be affected by the branched chain structure of the acid. Thus, while the enzyme system cholesterol:lecithin acyltransferase functions in bovine plasma (16), the proportion of phytanate in the form of cholesteryl ester is very small in relation to that present in phospholipids. Whether the lack of cholesteryl phytanate is due to the inactivity of the transferase towards the branched chain acid or to the absence of the phytanyl group from the 2-position of lecithin is not known.

It was observed that the proportion of phytanate in the total fatty acids of the plasma of lactating cows given silage was greater than in that of silage-fed steers. While the experimental conditions used in the two experiments differed, thus precluding a direct comparison, it is apparent from the results reported by Duncan and Garton (3) that lactation is associated with an increase in the proportion of phytanate in the plasma triglycerides and phospholipids of cows receiving grass-silage. This may be due, in part, to increased feed consumption associated with the nutritional demands of lactation. However, it is likely that phytanate will accumulate in plasma lipids when the availability of phytanate to the mammary gland is limited because mammary-gland lipoprotein lipase discriminates against phytanyl groups present in glycerides (13).

The apparent absence of phytanic acid from the plasma total fatty acids of two new-born calves (17) suggests that, in cows, the placental barrier is virtually impermeable to the multibranched acid. In a study of tissue lipids of the new-born lamb, Downing (18) was unable to detect any multibranched fatty acids, though there was evidence to suggest that some placental transference of monomethyl-branched acids had occurred.

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REFERENCES

1. Lough, A.K., in "Progress in the Chemistry of Fats and Other Lipids," Vol. 14, Editcd by R.T. Holman, Pergamon Press, Oxford, 1973, p. 1.
2. Dawson, R.M.C., N. Hemington, D. Grime, D. Lander, and P. Kemp, Biochem. J. 144:169 (1974).
3. Duncan, W.R.H., and G.A. Garton, Biochem. J. 89:144 (1963).
4. Klenk, E., and E.G. Kremer, Z. Physiol. Chem. 343:39 (1965).
5. Shorland, F.B., R.P. Hansen, and L.A.M. Prior, Proc. 7th Int. Congr. Nutr., Vol. 5, Pergamon Press (1966) p. 399.
6. Steinberg, D., J. Avigan, C.E. Mize, J.H. Baxter, J. Cammermeyer, H.M. Fales, and F.P. Highet, J. Lipid Res. 7:684 (1966).
7. Folch, J., M. Lees, and G.A.S. Stanley, J. Biol. Chem. 226:497 (1957).
8. Garton, G.A., W.R.H. Duncan, and A.K. Lough, Biochim Biophys. Acta 47:592 (1961).
9. Duncan, W.R.H., and G.A. Garton, J. Lipid Res. 3:414 (1962).
10. Hilditch, T.P., and P.N. Williams, "The Chemical Constitution of Natural Fats," 4th ed. Chapman and Hall, London, 1964 p. 510.
11. Robinson, D.S., in "Comprehensive Biochemistry," Vol. 18, Edited by M. Florkin and E.H. Stolz, Elsevier, Amsterdam, 1970 p. 87.
12. Scow, R.O., M. Hamosh, E.J. Blanchette-Mackie, and A.J. Evans, Lipids 7:497 (1972).
13. Laurell, S., Biochim. Biophys. Acta 152:80 (1968).
14. Ellingboe, J., and D. Steinberg, Biochim. Biophys. Acta 270:92 (1972).
15. Annison, E.F., J.L. Linzell, S. Fazakerley, and B.W. Nichols, Biochem. J. 102:637 (1967).
16. Noble, R.C., J.C. O'Kelly, and J.H. Moore, Biochim. Biophys. Acta 270:519 (1972).
17. Avigan, J., Biochim. Biophys. Acta 125:607 (1966).
18. Downing, D.T., J. Lipid Res. 5:210 (1964).

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